Preliminary study on factors influencing rabbit doe reproductive efficiency: Effect of parity, day of mating, and suckling on ovarian status and estrogen levels at day 6 of pregnancy

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Abstract

The rabbit corpus luteum becomes an estradiol-dependent tissue by day 6 of gestation, and adequate estrogen is critical to avoid pregnancy failure. The aim of this study was to investigate the effect of parity (primiparous or multiparous), day of mating (11 or 21 d postpartum), and suckling status (suckling or nonsuckling) on various reproductive traits in hybrid rabbit does (n = 96). Ovarian structures on day 6 after coitus were evaluated by means of ultrasonography. Blood samples were collected that day, and the serum was analyzed for estradiol-17β by radioimmunoassay (RIA). Parity and suckling had significant effects on mating rate ($P < 0.01$ and $P < 0.05$, respectively). More does accepted the male on day 11 than on day 21 ($P < 0.05$). Ovulation frequency was significantly affected by parity ($P < 0.05$), day of mating ($P < 0.01$), and suckling ($P < 0.01$). Fewer ovarian large follicles and lower estradiol-17β levels were detected in suckling compared with nonsuckling rabbits ($P < 0.01$). Since estrogen concentrations are commonly used to assess follicular growth and steroidogenic capacity, the lower hormonal levels in the suckling rabbits may reveal poorer ovarian activity, which could result in reduced reproductive efficiency. Our observations confirm the existence of a partial antagonism between lactation and reproduction in rabbits. Further research is needed to elucidate these phenomena, including when artificial insemination is done. Ultrasonography could represent a noninvasive and reliable method for studying several reproductive functions and dysfunctions in rabbits.

Résumé

Chez la lapine le corps jaune devient un tissu dépendant de l'œstradiol au 6e jour de la gestation, et une quantité adéquate d'estrogène est critique pour éviter l'interruption de la gestation. L'objectif de l'étude était d'examiner, chez des lapines hybrides (n = 96), l'effet de la parité (primipare ou multipare), le jour de l'accouplement (11 ou 21 jours post-partum), et si elles allaitaient ou non, sur différentes caractéristiques de reproduction. Les structures ovariennes au jour 6 après le coït ont été évaluées par échographie. Des échantillons de sang ont été prélevés la même journée et le sérum analysé par radio-immunoessai (RIA) pour l'œstradiol-17β. La parité et le fait d'allaiter avaient un effet significatif sur le taux de conception (respectivement $P < 0,01$ et $P < 0,05$). Plus de lapines étaient réceptives au mâle au jour 11 comparativement au jour 21 ($P < 0,05$). La fréquence d'ovulation était affectée de manière significative par la parité ($P < 0,05$), le jour de l'accouplement ($P < 0,01$) et le fait d'allaiter ($P < 0,01$). Moins de larges follicules ovariens et des niveaux plus faibles d'œstradiol-17β ont été trouvés chez les lapines qui allaitaient comparativement à celles qui ne le faisaient pas ($P < 0,01$). Étant donné que les concentrations d'œstrogène sont fréquemment utilisées pour évaluer la croissance folliculaire et la capacité de stéroidogénèse, les niveaux plus faibles d'hormones chez les lapines qui allaitent peuvent révéler une activité ovarienne plus pauvre, qui pourrait se traduire par une efficacité reproductrice moindre. Nos observations confirment l'existence d'un antagonisme partiel entre la lactation et la reproduction chez les lapins. De la recherche supplémentaire est nécessaire afin d'élucider ces phénomènes, incluant lorsque l'insémination artificielle est utilisée. Chez les lapins, l'échographie pourrait s'avérer une méthode non-invasive et fiable pour étudier plusieurs fonctions reproductrices ainsi que des dysfonctions.

(Traduit par Docteur Serge Messier)

Introduction

Control of the interval from parturition to subsequent conception is crucial to the optimal reproductive rate for a species. The remating program [interval from kindling (parturition) to mating] most widely used in the rabbit industry is the semi-intensive rhythm, which is 11 to 12 d postpartum. This should represent a compromise between the doe's need for recovering energy after parturition and the economic demand for increased numbers of kits weaned per year. Since a negative energy balance can be detrimental to the reproductive process (1), one of the main reasons for lengthening the remating interval is to prolong the dry period so that the doe, especially if primiparous, recovers body energy completely (2–4).

Ovulation does not occur spontaneously in rabbits: it is a neuroendocrine reflex triggered by copulation. Even though rabbit does can be mated just after kindling and be concurrently pregnant and lactating, their reproductive efficiency varies considerably with parity (the number of times the doe has kindled), physiological state

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(whether lactating or not and the stage of lactation), and sexual receptivity at mating (5). The current study aimed to investigate the effects on various aspects of rabbit reproduction of parity, suckling, and remating interval. The ovarian structures were examined by means of ultrasound scanning, which allowed the experiment to be conducted entirely *in vivo* and by a noninvasive procedure.

In previous studies of the relationship between different managerial strategies and rabbit ovarian function (ovulation response, ovulation rate, fertilization rate, embryo survival before and after implantation, pregnancy rate), the females were slaughtered and their reproductive tracts removed and dissected (6) or, alternatively, the ovaries were recovered by laparotomy (7). Even though ultrasonography has been used for pregnancy diagnosis (8), to characterize fetal growth (9), and to evaluate the maternal and fetal vessels (10), there is a paucity of information in the literature regarding specific study of the rabbit ovaries by this method.

In this work special focus was given to the ovarian population of large follicles (LF; diameter greater than 1.5 mm) present at day 6 of pregnancy because this follicular category, being steroidogenically active, should be crucial to survival of the developing corpora lutea (CL) and thus to reproductive efficiency. Blood estradiol levels in plasma were measured to evaluate the steroidogenic capability of the follicles through the first days of pregnancy.

Materials and methods

Animals and experimental design

The 96 hybrid rabbit does used in the study were housed in individual metal cages under controlled light conditions (14 h of light, 10 h of darkness), were fed *ad libitum* a commercial pelleted diet, and had free access to tap water. The protocols involving the care and use of the animals were approved by the Bioethics Committee of the University of Sassari, Sassari, Italy.

The 50 primiparous and 46 multiparous does were studied in a $2 \times 2 \times 2$ factorially designed experiment. The variables considered were parity, day of attempted mating [day 11 (n = 46) or day 21 (n = 50)] after parturition, and whether the doe suckled the litter after the day of parturition [S (n = 46)] or did not [N (n = 50)]. The time that a doe was exposed to a given male (of proven fertility) ranged from 60 to 120 s. If copulation occurred, the female was removed immediately and classed as mating. A nonmating doe was defined as one that had been exposed to 6 males for a total of about 12 min. Mating performance was assessed in the morning and after controlled lactation (1 period of suckling per day for 15 min).

The CL and follicle populations of the mated females (S and N) were evaluated by means of transabdominal real-time B-mode ultrasound scanning on day 6 after coitus, which corresponded to postpartum days 17 (groups 11-N and 11-S) and 27 (groups 21-N and 21-S). Blood samples were obtained from the central ear artery (11) on the same day and were processed to yield serum, which was stored at −20°C until assayed. The concentration of estradiol-17β (E_2) in the serum was determined by radioimmunoassay (RIA) with a commercial kit (Radim, Pomezia, Italy) based on competition between antigens labeled with iodine 125 (radioactive conjugate) and nonlabeled antigens (calibrator sample) for specific binding sites in antiserum-coated tubes. After incubation, all unbound material was removed and radioactivity measured. Uncoated tubes were prepared for measurements of total activity (T) and nonspecific binding (NSB). Tubes coated with rabbit antibody against E_2 were prepared for measurements in the zero calibrator (Bo), calibrators 1 to 6, control serum, and samples as follows. First, 100 µL of Bo was added to the NSB tube, and 100 µL of each additional calibrator as well as the control serum and samples was pipetted into the corresponding tube. Next, 500 µL of the radioactive conjugate was pipetted into all the tubes, whose contents were then mixed by vortex. After incubation for 3 h ± 5 min at 37°C ± 2°C the contents were carefully aspirated by pump from all tubes except the uncoated T tube. The radioactivity in the tubes was measured with a γ-counter. The sensitivity of the assay was 2 pg. The intra-assay coefficient of variation (CV) was 3%.

Scanning procedure

For ultrasound scanning the nonsedated rabbit does were put in the supine position. The abdominal skin was shaved at the beginning of the experiment, and before each scanning session the abdominal area was covered with scanning gel. The scans were done with a 10-MHz transducer (model UST-987.7.5; Aloka, Tokyo, Japan) with a real-time B-mode ultrasound scanner (SSD-900; Aloka). During each scanning session the scanner settings that affect image attributes (overall time gain, near-field and far-field gains, compensation, and beam focus) were kept at predetermined levels. The images were displayed at maximum magnification.

To scan the left and the right ovaries the rabbits were laid in a dorsolateral position. The ovaries were located by first identifying the kidney as a major landmark and then moving the transducer a few millimeters caudolaterally from the caudal edge of the kidney, remembering that the ovaries have a superficial location under the skin. The best technique was to start with the left ovary, which was in general easier to find. One or more on-screen images of both ovaries were studied to assess the follicular and luteal structures. To investigate the follicular populations, individual follicles on each ovary were identified, measured, and classified by diameter as small (≤ 1.5 mm) or large (> 1.5 mm). The total number of LF was calculated by counting those on both ovaries. The occurrence of ovulation was evaluated by the presence of CL, since rabbits are induced ovulators, and functional CL should not be present in the ovaries of unmated females. The ovulation rate was determined, as recommended by the International Rabbit Reproduction Group (12), by counting the number of CL on both ovaries.

Statistical method

Statistical analysis was done using SAS software, version 8.1 (SAS Institute, Cary, North Carolina, USA). A mixed procedure was used according to an autoregressive model to analyze repeated measures, including the effects of parity, remating interval, suckling, and their interactions on mating rate (number of does accepting the male/number of observed does × 100) (12), ovulation frequency (number of ovulating does/number of mated does × 100) (12), ovarian structures, and E_2 concentrations. Means were compared with a protected t-test; differences were considered significant when the P-value was less than 0.05.

Table I. Effects of parity, day of mating, suckling, and their interactions on reproductive traits of rabbit does

Reproductive trait or interaction	Mean ± standard error and level of statistical significance[a]				
	Mating rate (%)	Ovulation frequency (%)	Corpora lutea *(n)*	Large follicles[b] *(n)*	Estradiol-17β level (pg/mL)
Parity (P)					
Primiparous	66.0 ± 4.9**	79.7 ± 5.8*	8.9 ± 0.2	15.0 ± 1.5	14.5 ± 1.3
Multiparous	95.6 ± 6.7**	68.2 ± 4.5*	10.3 ± 0.8	16.2 ± 1.7	15.3 ± 1.5
Day of mating (D; number of days postpartum)					
11	86.8 ± 6.5*	69.6 ± 3.1**	9.0 ± 0.2	15.9 ± 1.3	14.9 ± 1.9
21	74.0 ± 5.1*	50.0 ± 3.9**	10.0 ± 0.7	15.3 ± 1.8	14.8 ± 1.6
Suckling status (S)					
Suckling	76.0 ± 5.9*	47.8 ± 3.7**	9.4 ± 0.9	12.1 ± 1.1**	12.6 ± 1.7**
Not suckling	88.1 ± 6.3*	68.1 ± 4.2**	9.9 ± 0.6	19.01 ± 2.2**	17.5 ± 2.1**
P × D	**	*	NS	NS	NS
S × P	NS	**	NS	NS	NS
D × S	*	*	NS	NS	NS

[a] Asterisks indicate a significant difference between a pair of means or a significant interaction at *P*-values of < 0.05 (*) or < 0.01 (**). The remaining pairs of means did not differ significantly. The interaction P × D × S was not significant.

[b] Those more than 1.5 mm in diameter.

NS — not significant.

Results

Results from the statistical analyses are depicted in Table I. Parity and suckling had significant effects on mating rate ($P < 0.01$ and $P < 0.05$, respectively). More does accepted a male on day 11 than on day 21 ($P < 0.05$). There were significant interactions between parity and day as well as between day and suckling with respect to mating rate. Ovulation frequency was depressed by suckling ($P < 0.01$) and was significantly affected by parity ($P < 0.05$), day of mating ($P < 0.01$), and the interactions parity × day, suckling × parity, and day × suckling.

The ovarian follicles generally appeared as anechoic wide circular areas on the ultrasound scans. However, when numerous within the same ovary they were sometimes flattened and packed together; in this situation there is a risk that the total number will be underestimated. There were fewer LF, accompanied by lower serum E_2 levels, in suckling does than in nonsuckling does ($P < 0.01$). In contrast, the numbers of LF and the serum E_2 levels were not influenced by parity or by day of mating. Data on LF number and E_2 levels referred to the rabbit does that ovulated. The number of CL, visible as moderately hyperechoic structures, was not affected significantly by parity, day of mating, suckling, or their interactions. Luteal structures were in some cases not clearly discernible because they appeared almost isoechoic with the ovarian parenchyma rather than hyperechoic.

Discussion

The higher percentage of rabbit does mating on day 11 than on day 21 postpartum is presumably consistent with greater sexual receptivity, and in fact the sexual behavior of does varies with the lactation stage. The percentage of does that accept a male is very high on the day of parturition, decreases at day 4 postpartum, increases at day 11 postpartum, and returns to the highest level after weaning (5). The primiparous lactating rabbits in our study had very poor mating performance on day 21 postpartum, the day of maximum milk production and lowest live body weight for primiparous rabbits (1): an energy deficit could have affected sexual receptivity.

Mating was not followed by ovulation in some does, both suckling and nonsuckling, in this study. However, in the primiparous subjects this lack of ovulation occurred only in the suckling group, which may indicate different effects exerted by suckling upon the hypothalamic centers regulating sexual receptivity and the release of luteinizing hormone (13). A possible relationship with parity requires further investigation.

The small difference in follicular population was not unexpected, since the rabbit doe maintains a relatively constant supply of preovulatory follicles. In domestic rabbits, in which there is no spontaneous gonadotropin surge, follicles undergo waves of continuous maturation, such that ovulable follicles are present at nearly any given time (14). The lower population of LF in the suckling group might be responsible for the lower serum E_2 levels of this group compared with the nonsuckling group. Since E_2 concentrations have been commonly used to assess follicular growth and steroidogenic capacity (15,16), the lower hormonal levels in the suckling rabbits may reveal poorer ovarian activity, which could result in reduced reproductive efficiency.

Ovulation frequency was negatively affected by suckling, which confirms that in nursing rabbits, as in other species, sexual receptivity and fertility after natural mating and artificial insemination appear to be depressed during the period of lactation. Indeed, these does are less fertile as a direct consequence of the lack of ovulation, fertility failure, or embryo death (5). The existence of a partial antagonism between lactation and reproduction, reflecting the corresponding hormonal antagonism between prolactin and the release

of gonadotropins in the rabbit, has been widely reported (17,18). In the current study prolactin was not assayed, but it is well known that secretion of this hormone is increased during lactation (19).

In this study, the serum E_2 levels were lower in suckling than in nonsuckling rabbits on day 6 after coitus. Estrogens have been shown to play a key role in the maintenance of CL in the rabbit, prompting Hilliard and Eaton (20) to refer to estradiol as the "ultimate luteotropin" in this species. The rabbit CL becomes an estradiol-dependent tissue by day 6 of pregnancy, as reported by Miller and Keyes (21), who demonstrated that adequate E_2 at day 6 of pregnancy and pseudopregnancy is critical to continued development of the CL. The luteotropic E_2 is supplied by ovarian LF, whose destruction leads to immediate failure of the CL and termination of pregnancy and pseudopregnancy (22–24).

Suckling had a depressive effect on both ovulation frequency and LF number in this study. Regarding the steroidogenic capability of the follicles present through the first days of pregnancy, early studies indicated that LF generally have a higher steroid content than small follicles and that the appearance of new LF may be reflected in the rise in serum E_2 concentration by day 4 to 6 after ovulation (20,25). Moreover, Mills et al (26) postulated a relationship between the estrogen secretion and the wave of follicle growth occurring in the first 6 d after mating. More recently Marongiu and Gulinati (27) used ultrasound scanning to inquire into this relationship after inducing ovulation by injection of human chorionic gonadotropin.

By performing transabdominal real-time ultrasonography, this trial demonstrated that rabbit ovaries can be evaluated *in vivo*. This noninvasive technique was found effective since the ultrasonic image allows immediate interpretation in most circumstances. Indeed, ultrasound scanning could represent a reliable method for studying several reproductive functions in the rabbit.

Since the ovulation response and fertility after artificial insemination are high in rabbits that accept mating and significantly lower when they are not receptive to the male (5), the data from the current experiment appear suitable to support additional research aimed to elucidate the same postpartum reproductive phenomena when artificial insemination is done. Nevertheless, further studies are necessary to clarify follicular growth patterns, overall ovarian dynamics, and uterus changes during simultaneous pregnancy and lactation, with the aim of optimizing reproductive efficiency through breeding systems adapted to rabbit doe physiology.

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